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NEW MOLECULARLY IMPRINTED POLYMERS GRAFTED ON SOLID SUPPORTS

Technical Field of the Invention

The present invention relates to a molecularly imprinted polymer, to a method for preparing said molecularly imprinted polymer, and to the use of said molecularly imprinted polymer.

Background Art

In the fields of medical, dietary, environmental and chemical sciences there is an increasing need for the selective separation of specific substances in complex mixtures of related substances. The end goal can be the preparative isolation of a certain compound or compounds or measurements of their concentration. Molecularly imprinted polymers (MIPs) often exhibit a high selectivity towards their substrate in analogy with the antibody-antigen complementarity.(1, 2) The technique shows promise in chiral separations of for example amino acid derivatives, peptides, phosphonates, aminoalcohols and beta-blocking compounds, affinity chromatography of nucleotides and the DNA-bases as well as substitute for antibodies in immunoassays for commercial drugs. Molecular imprinting (MI) consists of the following key steps:(1) Functional monomers are allowed to interact reversibly with a template molecule in solution. (2) The hereby formed template assemblies are copolymerised with a cross-linking monomer resulting in a cross-linked network polymer. (3) The template is displaced and the resulting MIP material can be used for selective molecular recognition of the corresponding compound. If the MIP material is crushed and sieved it can be packed in a chromatographic column and used for chromatographic separation of the template from structurally related analogs. Analytical as well as preparative applications are here possible. Preparative applications can be separation

of a compound from a complex mixture of structurally related compounds and isolation of the compound. This can be through an affinity chromatographic procedure where pH, ion strength or solvent gradients can be used in order to control the strength of interaction with the stationary phase. The separation can target enantiomers or diastereomers in a mixture of enantiomers or diastereomers of one or many compounds. Analytical applications can in addition to the above mentioned separations be: competetitive binding assays, chemical sensors or selective sample enrichments.

Currently the most widely applied technique to generate molecularly imprinted binding sites is represented by the non-covalent route developed by the group of Mosbach(3). This makes use of non-covalent self-assembly of the template with functional monomers prior to polymerisation, free radical polymerisation with a cross-linking monomer and then template extraction followed by rebinding by non-covalent interactions. Although the preparation of a MIP by this method is technically simple it relies on the success of stabilisation of the relatively weak interactions between the template and the functional monomers. Stable monomer-template assemblies will in turn lead to a larger concentration of high affinity binding sites in the resulting polymer. The materials can be synthesized in any standard equipped laboratory in a relatively short time and some of the MIPs exhibit binding affinities and selectivities in the order of those exhibited by antibodies towards their antigens. Most MIPs are synthesized by free radical polymerisation of functional monounsaturated (vinylic, acrylic, methacrylic) monomers and an excess of cross-linking di- or triunsaturated (vinylic, acrylic, methacrylic) monomers resulting in porous organic network materials. These polymerisations have the advantage of being relatively robust allowing polymers to be prepared in high yield using different solvents (aqueous or organic) and at different

temperatures (4). This is necessary in view of the varying solubilities of the template molecules.

The most successful non-covalent imprinting systems are based on commodity acrylic or methacrylic monomers, such as methacrylic acid (MAA), cross-linked with ethyleneglycol dimethacrylate (EDMA). Initially, derivatives of amino acid enantiomers were used as templates for the preparation of imprinted stationary phases for chiral separations (MICSPs) but this system has proven generally applicable to the imprinting of templates allowing hydrogen bonding or electrostatic interactions to develop with MAA.(5, 6) The procedure applied to the imprinting with L-phenylalanine anilide (L-PA) is outlined in Fig. 1. In the first step, the template (L-PA), the functional monomer (MAA) and the cross-linking monomer (EDMA) are dissolved in a poorly hydrogen bonding solvent (diluent) of low to medium polarity. The free radical polymerisation is then initiated with an azo initiator, commonly azo-N,N'-bis-isobutyronitrile (AIBN) either by photochemical homolysis below room temperature(6, 7) or thermochemically at 60°C or higher(5). Lower thermochemical initiation temperatures down to 40°C or 30°C may be obtained using azo-N,N'-bis-divaleronitrile (ABDV) and V70 resp. instead of AIBN as initiator (see).(7, 8) In the final step, the resultant polymer is crushed by mortar and pestle or in a ball mill, extracted by a Soxhlet apparatus, and sieved to a particle size suitable for chromatographic (25-38 μm) or batch (150-250 μm) applications.(6) The polymers are then evaluated as stationary phases in chromatography by comparing the retention time or capacity factor (k')(9) of the template with that of structurally related analogs.

As appears from above MIPs have sofar been prepared in the form of continuous blocks that need to be crushed and sieved before use. This results in a low yield of irregular particles, a high consumption of template and a material exhibiting low chromatographic efficiency. There

is therefore a need for MI-materials that can be prepared in high yield in the form of regularly shaped particles with low size dispersity and a controlled porosity. These are expected to be superior in terms of mass transfer characteristics and sample load capacity compared to the materials obtained from the monolithic approach.

Such MIPs have been previously prepared through suspension(10, 11)- polymerisation techniques, dispersion polymerisation(12) or precipitation polymerisation(13). This resulted in spherical particles of a narrow size distribution. These procedures have the limitation of being very sensitive to small changes in the manufacturing conditions and the type of solvents and polymerisation conditions that can be applied. Thus the procedures need careful optimization for each new template target which significantly reduces the usefulness of this route. Moreover conditions leading to low dispersity spherical particles may not be compatible with conditions leading to high selectivity and affinity for the template target.

An alternative to this procedure is the coating of preformed support materials.(14-16) MIPs have been prepared as grafted coatings on oxide supports(14, 16) on organic polymer supports(15) and on the walls of fused silica capillaries(17-19). The former technique allows the use of the wide variety of oxide support materials available with different sizes and porosities. Grafting techniques to prepare organic polymer coatings are expected to be generally applicable to molecular imprinting since the structure of the underlying support is already fixed. Thus compared to the large number of factors influencing the end result in suspension or precipitation type polymerisations a smaller number of factors is likely to influence the end result in the preparation of the imprinted coatings. This will make the grafted coatings techniques less sensitive to changes in conditions offering a more robust method. These types of coating techniqes are furthermore applicable to modify surfaces of

monolithic type supports or microchips prepared by lithographic techniques. The oxide based materials are rigid porous supports with a limited inner pore volume. An alternative support that could potentially carry more grafted imprinted polymer per unit weight and thus allow a higher density of imprinted sites would be to make use of swellable organic resins. In this context Merrifield resins containing grafted initiator or monomer could be used.

Sofar most imprinted coatings have been prepared by grafting polymers to the various surfaces. Thus the surface contains prior to polymerisation polymerizable double bonds that can add to the growing polymer chains in solution linking them to the surface. The problem with this technique is the presence of initiator in solution requiring the monomer mixture to be applied as a liquid thin film on the surface prior to polymerisation. Thus the exact amount of monomers that will coat the available surface with an up to ca 100 Å thick liquid film is dissolved together with initiator in an excess of solvent. Thereafter the modified support is added and the solvent evaporated to leave the monomer film and initiator on the surface. Polymerisation is then carried out usually at elevated temperatures. With this procedure the thickness of the polymer layer is difficult to control and capillary forces upon evaporation of solvent may cause incomplete wetting of the surface. Moreover a continuous method of synthesising the particles is difficult to envisage with this method.

A considerable improvement in this regard would be to confine the initiator radicals to the support surface (Fig. 2).(20, 21) In absence of chain transfer this would lead to chain growth occuring only from the surface of the support with no polymerisation occuring in solution. For molecular imprinting this would have important consequences. For instance the polymerisation can be carried out on the surface of initiator modified support particl-

es suspended in a mixture of the monomers and solvent. This would allow polymerisation in a simple tank reactor by either thermal or photochemical initiation. The latter technique would allow the particles to be modified during the sedimentation possibly leading to a continuous method for preparing the imprinted composite particles (Fig. 3). Polymerisation would here only occur on the particle surface leaving the solution containing the monomers unreacted. The monomer solution can thus be reused for the coating of several batches of particles. The problem of confining polymer chain growth to the support surface and supress it in solution can be solved by attaching the radical initiator so that the radical formed upon bond homolysis remains bound to the surface. Alternatively the radical formed that is not attached to the surface should undergo rapid reaction to give an unreactive species. It should be possible to prepare the grafted coatings using monomers such as those based on styren/divinylbenzene, methacrylates, acrylates, acrylamides and in the presence of one or more template molecules.

Summary of the Invention

Thus, the present invention relates to a molecularly imprinted polymer obtainable by polymerising a composition comprising at least one monomer, and a template, on a support in a polymerisation medium with a free radical initiator, whereafter the template is removed from the molecularly imprinted polymer obtained, said polymerisation being confined to the surface of the support.

The invention further relates to a method for preparing a molecularly imprinted polymer which comprises polymerising a composition comprising at least one monomer, and a template, on a support in a polymerisation medium with a free radical initiator, whereafter the template is removed from the molecularly imprinted polymer obtained, said polymerisation being confined to the surface of the support.

Still further the invention relates to the use of a molecularly imprinted polymer as defined above in chromatography, for separations, in chemical sensors, in molecular recognition as stationary phase in capillaries, in selective sample enrichment or in catalysis.

These and other advantages and characterising features of the present invention will appear from the following specification and the appended claims.

Brief Description of the Drawings

Fig. 1 illustrates molecular imprinting with L-phenylalanine anilide (L-PA).

Fig. 2 illustrates the procedure of confining initiator radicals to the surface of a support.

Fig. 3 illustrates a method for preparing imprinted composite particles.

Fig. 4A illustrates the use of a presynthesized azo-silane initiator where both ends may be attached to the surface of a support.

Fig. 4B illustrates an initiator that may be preadsorbed on a support surface and that is insoluble in the monomer containing solution.

Fig. 4C illustrates the use of microwaves to selectively heat the particle surface.

Fig. 4D illustrates the use of iniferters such as dithiocarbamate coupled onto the surface.

Detailed Description of the Invention

The invention will now be described in more detail with reference to a number of non-limiting examples:

The invention refers to a material that consists in a support (porous or nonporous material or planar surface) coated with a polymer layer, a method for its fabrication and use of said material in for instance chromatography, for separations, in chemical sensors, in selective sample enrichment, in molecular recognition as stationary phase in capillaries or in catalysis. The material is prepared by grafting a polymer layer on the surface of a preformed organic or inorganic support material or surface. The grafting can be combined with the technique of molecular imprinting.

In one embodiment of the present invention the polymerisation is confined to the surface of the support by confining the free radical initiator to the support. According to one aspect the free radical initiator is bound (covalently or non-covalently such as e.g. by hydrogen bonds) to the surface of the support. According to another aspect the free radical initiator is adsorbed to the surface of the support, preferably by dissolving it in a solvent for the free radical initiator, applying the solution to the support, and removing the solvent, said free radical initiator being insoluble in the polymerisation medium or remaining attached to the support surface by adsorptive forces.

In another embodiment of the present invention the polymerisation is confined to the surface of the support by subjecting the composition, the support and the free radical initiator to microwave irradiation which selectively heats the support and thereby initiates a polymerisation reaction at the surface of the support.

In a further embodiment of the present invention the polymerisation is repeated at least once with a different composition to obtain at least one further layer of molecularly imprinted polymer. This allows the manufacturing of layered surfaces containing one or more imprinted layers using possibly different templates and layers of different polarity or other functional properties.

The support used in the present invention is preferably selected from the group consisting of porous and non-porous, planar and non-planar inorganic and organic supports. As examples of such support materials may be mentioned oxides such as alumina and silica, and organic resins in the form of particles such as spheres, or sheets.

The template used in the present invention may be any molecule or ion and is preferably selected from the group consisting of organic or inorganic molecule entities, ions, antibodies, antigens, amino acids, peptides, proteins, nucleotides, DNA-bases, carbohydrates, drugs, pesticides, and derivatives thereof, etc.

The expression "polymerisation medium" as used herein means a liquid medium in which the polymerisation is carried out. The polymerisation medium may e.g. be a solvent in which the monomers are soluble. It may also be a monomer acting as a solvent for the other components of the polymerisable composition.

The support surface is prepared as follows. A free radical initiator is bound to the surface either covalently or noncovalently so that the free radicals generated upon initiation remain confined to the surface or vicinity of the surface. The absence of polymer propagation in solution will lead to a higher accessibility of the monomers at the surface. Furthermore this method will allow the tuning of the thickness of the polymer layer.

Surface attachment of a free radical initiator has been disclosed generally by Guyot et.al. (21) and Tsubokawa et.al.(22, 23) It relies on presilanization of the surface using 3-aminopropyltriethoxysilane or a glycidoxypropylsilane (GPS) followed by reaction of the amino groups or the epoxy groups with an azoinitiator such as azo-bis(cyanopentanoic acid, ACPA) leading to the formation of an amide (using DCC as condensing reagent) or ester link between the surface and the azoinitiator. Also peroxy initiators may be used although better re-

sults are obtained using the grafted azoinitiator followed by photochemical initiation. High yields of grafted polymer are obtained using silica reacted with toluene-2,4-diisocyanate (TDI) followed by reaction with ACPA.

Example 1

Coupling of initiator to amino, epoxy or chloromethyl modified supports or resins

Epoxy and chloromethyl modified supports: A typical example is as follows. Into a flask, 3 g of epoxy modified particles 50 mL of DMSO, 0.5 g of ACPA and picoline were charged. The reaction mixture was stirred for 5 h at 50°C. After the reaction the particles were washed with methanol and dried.

Amino modified supports: A typical example is as follows. Into a flask, 3 g of epoxy modified particles 50 mL of DMF, 0.5 g of ACPA and dicyclohexyldicarbodiimide (DCCI) and base were charged. The reaction mixture was stirred for 5 h. After the reaction the particles were washed with methanol and dried.

The above procedure does not confine all initiator radicals to the surface since the initiator is bound at only one position. This invention describes three alternative procedures to confine the polymerisation to the surface.

1. The use of a presynthesized azosilane (Fig. 4A). This will more likely lead to a two point attachment of the initiator to the surface.

Example 2

Synthesis of azosilane for two point coupling of an azo-initiator to a surface or support

The azosilane was synthesized by mixing 0.5 mole glycidoxypropyltrimethoxysilane (GPS) and 0.25 mole ACPA in 200 mL isopropanol and catalytic amounts of picoline. The reaction was allowed to continue at room temperature and the product isolated by evaporation to dryness followed by purification by column chromatography giving the product in 60 % yield.

Example 3

Coupling of silane to a surface

The silane was coupled by reaction in water at low temperature (20°C) for 24 hours.

2. Preadsorbtion of an initiator that is insoluble in the monomer containing solution. Thus, a polar water soluble initiator as for instance an azo-bis-amidine,(24) can be adsorbed to the surface from aqueous solvent, the surface dried and then the polymerisation initiated as described above (Fig. 4B). The free radicals generated from the initiator will stay associated to the surface due to their insolubility in the monomer mixture.

Example 4

Adsorption of amidineazoinitiator to a support surface

An amidineazoinitiator such as 2,2′-azobis(N,N′-dimethyleneisobutyramidine) or 2,2′-azobis(2-amidino-propane) is dissolved in methanol/water and support particles such as silica are added. After several hours of equilibration the solvent is removed by filtration and the particles dried under vacuum.

3. Use of microwaves to selectively heat the particle surface (Fig. 4C).

Example 5

Microwave initiated polymerisation

Particles are added to a solution of monomers and initiator in a suitable solvent. The polymerisation is initiated by microwave irradiation at a wavelength causing local heating of the particles only.

4. Use of iniferters such as dithiocarbamate coupled onto the surface (Fig. 4D).(25) (The term "iniferter" is an abbreviation for "initiator + transfer agent + terminator").

Example 6

Synthesis of support or polymeresin bound initiator

To a surface or polymer containing bound chloromethyl groups is given N,N-diethyldithiocarbamate in solution and the reaction allowed to proceed at elevated temperatures.

Example 7

Synthesis of block-graft imprinted copolymer

Particles or a surface containing bound dithiocarbamate groups are/is added to a mixture of monomers (concentration about 5 moles/litre), template and solvent under nitrogen. The polymerisation was initiated by irradiation with an ultrahigh pressure mercury UV lamp and allowed to proceed for a certain time. Then the unreacted monomers and template were washed away. The obtained particles or surface can then be immersed in another solution containing another monomer and the procedure repeated. This allows the manufacturing of layered surfaces containing one or more imprinted layers using possibly different templates and layers of different polarity or other functional properties.

Example 8

Endcapping of unreacted silanol groups

Prior to polymerisation endcapping of unreacted silanol groups can be done. Hexamethylsilazane is here effective. Good wetting is critical for the formation of a homogenous layer fully covering the support. Another possibility to enhance the wetting is to use organosilanes containing functionalities resembling solvents known to be good solvents for the methacrylate polymerisations. Among these chlorinated hydrocarbons are particularly useful.

Grafting of polymer layer

The polymerisation can be carried out in a stirred suspension of the particles in the monomer mixture since growth only takes place on the surface (see Fig. 3). Thus the initiator modified particles are added to a

monomer containing solution and solvent and possibly a template and the suspension stirred. The polymerisation is then carried out photochemically or thermally. The particles can be based on any inorganic or organic support material and the template on any molecule or ion dissolved in the monomer mixture solution. The grafting can also occur on other surfaces such as those generated by lithographic processes or on the walls of capillaries or fibres. The thickness of the polymer layer is tunable by varying the time of reaction.

<u>Example</u>

To a stirred solution of 38 ml (0.2 mole) EDMA, 3.4 ml (40 mmole) MAA and 10 mmole terbutylazine (or no template) in 56 ml dichloromethane is added 5 g of any of the initiator modified particles described in Examples 1-6. The suspension is sparged with nitrogen and the polymerisation initiated by UV irradiation using a standard high pressure mercury lamp at 15°C or by heating to a temperature providing a suitable rate of polymerisation. The suspension is stirred under nitrogen and UV irradiation or heating for 24 h and the particles then filtered, washed and dried under vacuum. The monomer mixture is then used to modify a second batch of particles.

The resulting particles exhibit high selectivity and affinity for the template, terbutylazine.

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